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METHODS OF SAMPLING AND TEST FOR PROCESSED FRUITS AND VEGETABLES

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

Gr 8 *January* 1965

Indian Standard

METHODS OF SAMPLING AND TEST FOR PROCESSED FRUITS AND VEGETABLES

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AMENDMENT NO. 1 FEBRUARY 1995
TO
IS 2860 : 1964 METHODS OF SAMPLING AND TEST FOR PROCESSED FRUITS AND VEGETABLES

(*Page* 4, *Table* 1) — Add the following as 'Column 4':

'ACCEPTANCE NUMBER (c)
(4)
0
1
1
2
2
3
3'

(*Page* 6, *clause* 3.4.5) — Add the following new clause after 3.4.5:

'3.4.6 The lot shall be declared as acceptable for organoleptic characteristics, such as colour, flavour, defects, size and appearance, if the maximum number of defectives in the sample does not exceed the permitted acceptance number (c) given in col 4 of Table 1.'

(FAD 10)

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Indian Standard

METHODS OF SAMPLING AND TEST FOR PROCESSED FRUITS AND VEGETABLES

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 19 September 1964, after the draft finalized by the Fruits and Vegetables Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Different methods of analysis are at present being followed in different laboratories for testing the processed fruits and vegetables. Owing to differences in the methods employed, there have been often differences of opinion about the quality of the consignments. With the adoption of the methods prescribed in this standard, it is expected that differences in the results due to adoption of different methods in different laboratories will be reduced.

0.3 In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960*.

1. SCOPE

1.1 This standard prescribes the methods of sampling and test for processed fruits and vegetables.

2. QUALITY OF REAGENTS

2.1 Pure chemicals and distilled water (*see* IS : 1070 - 1960†) shall be employed in tests. Where a reagent of a special quality is required, it has been specified in the detailed method.

NOTE — ' Pure chemicals' shall mean chemicals that do not contain impurities which affect the experimental results.

3. SAMPLING

3.1 General Requirements of Sampling

3.1.1 Sampling shall be done by a person agreed to between the purchaser and the vendor and if desired by any of them, in the presence

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*Rules for rounding off numerical values (*revised*).

†Specification for water, distilled quality (*revised*).

of the purchaser (or his representative) and the vendor (or his representative).

3.1.2 Samples shall be stored in such a manner that the temperature of the material does not vary unduly from the normal temperature.

3.2 Scale of Sampling

3.2.1 *Lot* — In any consignment, all the cans of the same size containing material of the same type, style, and grade shall constitute a lot.

3.2.1.1 Samples shall be examined from each lot separately for ascertaining the conformity of the material to the respective specifications.

3.2.2 *Selection of Sample* — The number of packing cases to be selected from a lot for drawing the sample cans shall depend on the size of the lot and shall be in accordance with col 1 and 2 of Table 1.

TABLE 1 SCALE OF SAMPLING

No. of Cans in the Lot (1)	No. of Packing Cases to be Opened (2)	No. of Cans to be Selected (3)
Up to 200	3	6
201 „ 300	4	8
301 „ 500	5	10
501 „ 800	6	12
801 „ 1 300	7	14
1 301 „ 3 200	8	16
3 201 and above	10	20

3.2.3 The packing cases shall be chosen at random from the lot and for this purpose some random number table as agreed to between the purchaser and the vendor shall be used. In case such a table is not available, the following procedure shall be adopted.

3.2.3.1 Arrange all the packing cases in a systematic manner and count them as 1, 2, 3............etc, up to r and so on. Every rth case so counted shall be withdrawn,

r being the integral part of $\frac{N}{n}$

where

N = total number of cases in the lot, and

n = number of cases to be chosen.

If *r* turns out to be a fractional number, its value should be taken as equal to its integral part.

3.2.4 From each of the packing cases selected (**3.2.3.1**), two cans shall be drawn at random so as to get the total number of cans from the lot as shown in col 3 of Table 1.

3.2.5 In addition to the cans selected as in **3.2.4**, select 8 cans at random as far as possible from all the cases selected (**3.2.3**) for testing the microbiological requirements.

3.3 Testing of Samples

3.3.1 Each of the cans, selected as in **3.2.4**, shall be tested individually for all the requirements as laid down in the respective specifications, except the requirements for metallic impurities (*see* **3.3.2**) and the microbiological requirements (*see* **3.3.3**).

3.3.2 After testing the sample cans (**3.2.4**) for individual characteristics, the contents of all the cans shall be mixed to form a composite sample and the composite sample so formed shall be tested for arsenic, lead, copper, zinc, tin and calcium.

3.3.3 *Tests for Bacteriological Requirements*

3.3.3.1 *Incubation at 37°C* — Half of the cans selected as in **3.2.5** shall be incubated at 37°C for not less than 7 days and subjected to bacteriological examination.

3.3.3.2 *Incubation at 55°C* — The remaining half of the cans shall be incubated at 55°C for not less than 7 days and subjected to bacteriological examination.

NOTE — In the case of processed fruits, the cans shall be incubated only at 37°C.

3.4 Criteria for Conformity

3.4.1 *For Vacuum and Head Space Requirements* — The lot shall be declared as conforming to the requirements for vacuum and head space, when each of the sample cans (**3.2.4**) tested individually satisfies the requirements given in the individual specification for the material.

3.4.2 *For Drained Weight* — The average of the results of drained weight of each of the cans tested (**3.2.4**) shall satisfy the requirement given in the individual specifications.

3.4.3 *For Syrup and Brine* — The test results for the characteristics of syrup or brine for each of the sample cans (**3.2.4**) tested, shall satisfy the requirements given in the individual specifications.

3.4.4 *For Metallic Impurities* — The test results for the heavy metals on the composite sample (**3.3.2**) shall satisfy the requirements prescribed for arsenic, lead, copper, zinc and tin.

3.4.5 *For Microbiological Requirements* — For declaring the conformity of the lot to the microbiological requirements, the test results (**3.3.3.1** and **3.3.3.2**) shall satisfy the requirements of the test.

4. EXAMINATION OF THE CAN

4.1 Exterior Condition — Examine the sample cans (**3.2.4**) for dents, rust, perforations, seam distortions, etc, after removing the labels. Mark the cans with a code number. The condition of the can shall also be examined for any leaking, panelling or swelling.

4.2 Interior Condition — After determining the drained weight (*see* **7**), wash the can, clean and examine for any black discolouration marks, rusting and pitting. Also examine the condition of the can.

5. DETERMINATION OF VACUUM OF THE CANS

5.1 The vacuum in the cans shall be determined with an electric recording type machine without opening the can. If such a machine is not available, a vacuum gauge of the piercing type shall be used. Report vacuum in millimetres of mercury. Necessary corrections for altitude may be made.

6. DETERMINATION OF HEAD SPACE

6.0 General — Either of the two methods, namely, Method 1 (*see* **6.1**) and Method 2 (*see* **6.2**) may be used. Method 1 shall be the referee method in case of dispute.

6.1 Method 1

6.1.1 *Apparatus*

6.1.1.1 *Rotary cutter*

6.1.1.2 *Measuring stick* — a clean straight flat piece of wood of suitable type not more than 3·2 mm in thickness and 6·4 mm in width.

6.1.2 *Procedure* — Cut out the lid on the edge of the end-plate partially by the rotary cutter, and lift the cut portion carefully so that the shape of the end-plate is not altered. Introduce the measuring stick in the can and make certain that it goes straight in and rests flat against the bottom of the can and against the body plate. Rest for a short time and lift the stick straight. Find out the length of the stick which is wet, the space occupied by the contents of the can. Empty out the contents of the can carefully and fill it completely with water. Again introduce a similar measuring stick and find out the length of the stick which is wet. The difference between the readings gives the gross head space. Net head space is equal to gross head space less 5 mm. Take four recordings. The average of the four net

head space recordings taken at different points shall be the head space of **the can.**

6.2 Method 2

6.2.1 *Apparatus*

6.2.1.1 *Rotary cutter*

6.2.1.2 *Ruler* — with millimetre divisions.

6.2.2 *Procedure* — Cut out the lid on the edge of the end-plate with the **rotary cutter, and** lift the cut portion carefully so that the shape of the end-**plate is not altered.** Take the measurement from the top of the double **seam to the surface** of the liquid using a ruler as illustrated in Fig. 1. The **average of four** measurements taken at different points shall be the head **space of the can.**

Fig. 1 Measurement of Head Space in Cans

7. DRAINED WEIGHT

7.1 Apparatus

7.1.1 *1·70-mm IS Sieve (Aperture 1·70 mm)* — Alternatively, BS Sieve 10 or ASTM Sieve 12 or Tyler Sieve 10 may be used as they are equivalent to 1·70-mm IS Sieve (*see* IS : 460-1962*).

7.1.1.1 For determining the drained weight in case of tomatoes, 4·75-mm IS Sieve shall be used.

7.2 Procedure — Carefully weigh the clean and dry sieve and empty the contents of the can into the sieve in such a manner as to distribute the product evenly. Without disturbing the product, incline the sieve so as to facilitate drainage. Allow to drain for two minutes, and weigh the sieve again along with the product. The drained weight shall be the weight of the product and sieve less the weight of the sieve.

7.2.1 A sieve of size 20·3 × 20·3 cm shall be used for A-2½ cans or smaller. In case of cans of size larger than A-2½, a sieve of size 30·5 × 30·5 cm shall be used.

8. DETERMINATION OF *p*H OF CUT-OUT SYRUP

8.1 Determination of *p*H — The *p*H of the cut-out syrup shall be determined by either the colorimetric (comparator) method or the potentiometric method.

8.1.1 *Colorimetric (Comparator) Method* — The method is based on the fact that certain indicators show definite changes of colour, depending on the *p*H of the sample tested. Accuracy higher than 0·2 *p*H unit may not be expected from this method.

Various models are available in the market for the colorimetric determination of *p*H consisting basically of a comparator, standard disc to be used with each indicator; standard indicator solution, 1-ml graduated pipette and cells or test-tubes in duplicate. The detailed instructions for making the test are furnished with each set which shall be followed. Usually, 10 ml of the syrup is used and 0·2 ml of a standard indicator solution is added. Another cell containing the syrup without indicator solution serves as a control for matching the colour. The blank tube is placed on the left, and the syrup containing the indicator on the right in the slots provided in the comparator. The standard disc is rotated to get an even match. The determination is carried out in the daylight or with good daylight lamp. The indicators most suitable for determining the *p*H of syrups colorimetrically are methyl orange (*p*H 3·1 to 4·4), methyl red (*p*H 4·3 to 6·3) and bromocresol purple (*p*H 5·4 to 7·0).

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*Specification for test sieves (*revised*).

8.1.2 *Potentiometric Method* — This is the most accurate and reliable method for measuring *p*H. Several types of apparatus are available for the electrometric measurement either working with the help of dry cells, battery (bench types) or directly from the mains, where DC supply is also available. With each instrument, the instructions of the maker shall be followed.

8.1.2.1 *Apparatus*

a) *Syrup half cell* — Place in a 25-ml beaker or a wide mouth test-tube enough syrup to cover the glass electrode.

b) *The standard saturated calomel half cell* — The calomel electrode consists of a layer of purified mercury, covered with a mixture of mercury and calomel, above which is a saturated solution of potassium chloride saturated with calomel. The mixture of mercury and calomel is prepared by grinding them into a paste with a pestle and mortar. The mixture is washed with saturated potassium chloride solution by decantation several times. It is then shaken with the remainder of potassium chloride solution. When saturated with calomel, the solution is decanted and kept in a stoppered bottle for further use.

There are many forms of electrode vessels available to set up the half cell. The connection between the mercury and the potentiometer is made by a platinum wire. The two half cells are conveniently connected by an agar-agar bridge (thin glass tube bent into a U-shape and filled with agar solution containing 5 g of agar-agar per 100 ml of saturated potassium chloride solution). The bridge shall be kept in a saturated potassium chloride solution when not used.

8.1.2.2 *Procedure* — Standardize the instrument against a buffer solution of known *p*H and check against another buffer of different *p*H. Then connect the saturated calomel and syrup half cells to the potentiometer. Balance the potentiometer to take the reading.

Equilibrium occurs almost instantaneously and no drift is experienced. The *p*H is indicated directly on the instrument.

NOTE — When a batch of samples is to be examined, remove the agar-agar bridge after testing one sample, wash the end dipping into the syrup carefully with glass-distilled water and replace in the next sample.

9. DETERMINATION OF SPECIFIC GRAVITY

9.1 Apparatus

9.1.1 *Specific Gravity Bottle*

9.2 Procedure — Clean and thoroughly dry the specific gravity bottle and weigh it. Fill it up to the mark with freshly boiled and cooled water, which has been maintained at a temperature of 20° ± 1°C and weigh.

Remove the water, dry the bottle again and fill it with the material maintained at the same temperature. Weigh the bottle again.

9.3 Calculation

9.3.1 Specify the temperature of testing.

9.3.2 Calculate as follows:

$$\text{Specific gravity at } 20°/20°\text{C} = \frac{C - A}{B - A}$$

where

C = weight in g of the specific gravity bottle with the material,

A = weight in g of the empty specific gravity bottle, and

B = weight in g of the specific gravity bottle with water.

9.3.2.1 To find out the degrees Brix, the table given in Appendix A shall be used.

10. ACIDITY OF CUT-OUT SYRUP

10.1 Apparatus

10.1.1 *Erlenmeyer Flask* — of 250 ml capacity.

10.2 Reagents

10.2.1 *Standard Sodium Hydroxide Solution* — 0·1 N.

10.2.2 *Phenolphthalein Indicator Solution* — prepared by dissolving 0·1 g of phenolphthalein in 100 ml of 60 percent rectified spirit (*see* IS : 323-1959*).

10.3 Procedure — Weigh 10 g of the syrup into the Erlenmeyer flask, add a few millilitres of water and mix thoroughly. Titrate against the standard sodium hydroxide solution using 0·3 ml phenolphthalein indicator solution till a faint pink colour, persisting for 30 seconds, appears. Calculate the percentage acidity in terms of citric acid from the relationship, 1 ml of 0·1 N sodium hydroxide is equivalent to 0·006 4 g of citric acid anhydrous.

11. DETERMINATION OF SODIUM CHLORIDE IN BRINE

11.0 General — Either of the two methods, namely, Method 1 (*see* **11.1**) and Method 2 (*see* **11.2**) may be used. Method 1 shall be the referee method in case of dispute.

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*Specification for rectified spirit (*revised*).

11.1 Method 1

11.1.1 *Apparatus*

a) *Graduated flask* — of 100 ml capacity.

b) *Erlenmeyer flask* — of 300 ml capacity.

11.1.2 *Reagents*

a) *Ethyl alcohol*

b) *Concentrated nitric acid*

c) *Standard silver nitrate solution* — 0·1 N.

d) *Ferric alum indicator solution* — a saturated solution of ferric ammonium sulphate [$FeNH_4(SO_4)_2, 12H_2O$].

e) *Standard ammonium thiocyanate solution* — 0·1 N.

11.1.3 *Procedure* — Weigh exactly 5·0 g of the brine and transfer to a 100-ml graduated flask with roughly 50 ml of 80 percent alcohol. Shake well to suspend all insoluble material. Add 1 ml of concentrated nitric acid, and with pipette add excess of known volume of 0·1 N silver nitrate solution. Dilute to 100 ml with alcohol. Then transfer the mixture to the centrifuge bottle and centrifuge for five minutes at approximately 1 800 rev/min.

Pipette 50 ml of the supernatant liquid into a 300-ml Erlenmeyer flask and to it add 2 ml of concentrated nitric acid and 2 ml of ferric alum indicator solution. Titrate with the standard ammonium thiocyanate solution to a permanent light brown colour.

11.1.4 *Calculation* — Sodium chloride, in the brine, percent by weight

$$= \frac{11{\cdot}6\,(V_1N_1 - V_2N_2)}{W}$$

where

V_1 = volume in millilitres of the standard silver nitrate solution,

N_1 = normality of the standard silver nitrate solution,

V_2 = volume in millilitres of the standard ammonium thiocyanate solution used,

N_2 = normality of the standard ammonium thiocyanate used, and

W = weight in g of the brine in the aliquot.

11.2 Method 2

11.2.1 *Reagents*

a) *Standard sodium hydroxide solution* — 0·1 N.

b) *Standard silver nitrate solution* — 0·1 N.

c) *Phenolphthalein indicator solution* — prepared by dissolving 0·1 g of phenolphthalein in 100 ml of 60 percent rectified spirit (*see* IS : 323-1959*).

d) *Potassium chromate indicator solution* — approximately 5 percent (*w/v*).

11.2.2 *Procedure* — Take a suitable aliquot of the solution prepared as in **11.1.3**. If it is acidic, neutralize it with the standard sodium hydroxide solution using phenolphthalein as an indicator. Then titrate with the standard silver nitrate solution using potassium chromate solution as an indicator.

11.2.3 *Calculation* — Sodium chloride, in the brine, percent by weight

$$= \frac{5{\cdot}8\ (VN)}{W}$$

where

V = volume in ml of the standard silver nitrate solution used,
N = normality of the standard solution, and
W = weight in g of the brine in the aliquot.

12. DETERMINATION OF CALCIUM

12.1 Reagents

12.1.1 *Hydrochloric Acid* — 0·1 N.

12.1.2 *Sodium Acetate Solution* — 20 percent.

12.1.3 *Sodium Oxalate Solution* — saturated.

12.1.4 *Dilute Sulphuric Acid* — one volume of concentrated sulphuric acid (sp gr 1·84) diluted with 4 volumes of water.

12.1.5 *Standard Bromocresol Green Indicator Solution* — prepared by dissolving 0·1 g of bromocresol green in 100 ml of rectified spirit (*see* IS : 323-1959*).

12.1.6 *Standard Potassium Permanganate Solution* — 0·05 N.

12.1.7 *Acetic Acid*

12.2 Procedure

12.2.1 Weigh accurately about 25 g of the composite sample (**3.3.2**) in a previously weighed 50 to 100 ml dish. Heat the dish at 100°C until water is expelled, and continue heating slowly until the swelling stops. Place the dish in a closed muffle furnace at 525° ± 20°C and leave until white ash is obtained. Treat the ash with 25 ml or more of 0·1 N

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*Specification for rectified spirit (*revised*).

hydrochloric acid and heat to incipient boiling on an asbestos plate till all the soluble constituents have completely dissolved. Cool and filter through a Whatman No. 1 filter paper into a 250-ml graduated flask.

12.2.2 Take a 50 to 100 ml aliquot of the solution obtained in **12.2.1** in a 400-ml beaker and add 8 to 10 drops of bromocresol green indicator solution and then the sodium acetate solution till the colour of the solution is distinctly blue. Adjust the *p*H of the solution at 4·4 to 4·6 adding acetic acid drop by drop until the colour changes to distinct green. Filter the solution and bring it to boil. While still hot, add the saturated sodium oxalate solution dropwise as long as any precipitate forms, and then add an excess, and keep on boiling water-bath for one hour. Allow to stand for 3 hours or longer, preferably overnight. Decant the clear solution through a quantitative filter paper. Pour 15 to 20 ml of hot water on the precipitate and again decant the clear solution. Dissolve any precipitate remaining on the filter paper by washing with hot dilute hydrochloric acid into the original beaker; wash the filter paper thoroughly with hot water. Then reprecipitate, while boiling hot, by the addition of sodium acetate and a little of the saturated sodium oxalate solution. Allow to stand for 3 hours or more as before, filter through the same filter, and wash with hot water until it is chloride free.

12.2.3 Perforate the apex of the filter cone, wash the precipitate with 50 ml of hot water into the beaker used for precipitation. Add roughly 10 ml of dilute sulphuric acid, heat to 90°C and titrate with the standard potassium permanganate solution.

12.3 Calculation — Calculate as percent calcium. One millilitre of 0·05 N potassium permanganate solution is equivalent to 0·001 g of calcium.

13. DETERMINATION OF ARSENIC

13.1 Apparatus (Gutzeit Bottle) — Assemble the apparatus shown in Fig. 2 as follows:

> Take a 60-ml wide mouth bottle. Equip it by means of a perforated stopper with a glass tube *A* measuring 120 × 10 mm with a constriction at a distance of 50 mm from the upper end. Place a small wad of glass wool in the constricted bottom end of the tube and add about 4 g of clean dry sand. Moisten the sand with lead acetate solution (10 percent *w*/*v*) and remove the excess by light suction. Place dry lead acetate paper in the lower portion of the tube for the removal of hydrogen sulphide. Connect the tube by means of a rubber stopper with a narrow glass tube *B*, measuring 100 × 2·7 mm with a constriction at a distance of 60 mm from the upper end. Place in this tube the strip of mercuric bromide paper (**13.2.1**).

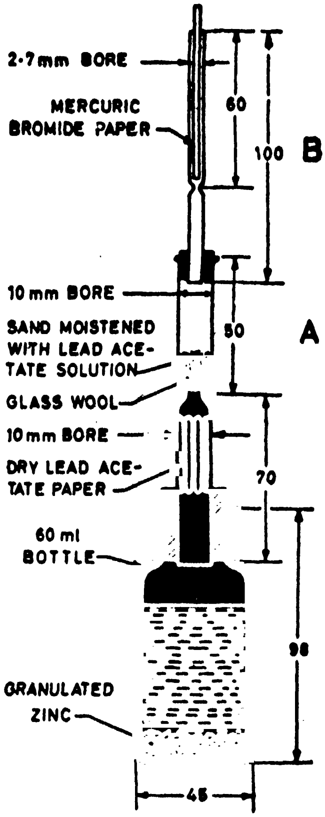

Fig. 2 Apparatus for Determination of Arsenic

13.2 Reagents

13.2.1 *Mercuric Bromide Paper* — Prepare by cutting paper Whatman No. 40, or its equivalent filter paper, into strips 2·5 mm wide and 120 mm long and soaking the strips for one hour in a fresh 5 percent solution of mercuric bromide in 95 percent ethyl alcohol. Dry and store in a dark bottle covered outside with black paper. Use the strips as soon as possible.

13.2.2 *Concentrated Nitric Acid* — sp gr 1·42.

13.2.3 *Concentrated Sulphuric Acid* — sp gr 1·84.

13.2.4 *Concentrated Hydrochloric Acid* — sp gr 1·16.

13.2.5 *Potassium Iodide Solution* — 15 percent (*w/v*)

13.2.6 *Stannous Chloride Solution* — Dissolve 40 g of arsenic-free stannous chloride ($SnCl_2$, $2H_2O$) in hydrochloric acid and make up the volume to 100 ml with hydrochloric acid.

13.2.7 *Granulated Zinc* — arsenic-free.

13.2.8 *Standard Stock Solution of Arsenic* — Dissolve 1·320 g of arsenic trioxide (As_2O_3) in 25 ml of sodium hydroxide solution (20 percent *w/v*). Saturate the solution with carbon dioxide and dilute to 1 000 ml with recently boiled water. One millilitre of this solution contains one milligram of arsenic.

13.2.9 *Standard Dilute Solution of Arsenic* — Dilute 40 ml of the stock solution (**13.2.8**) to 1 000 ml. Mix thoroughly. Take 50 ml of the diluted solution and dilute it further to 1 000 ml. This solution contains 0·002 mg of arsenic per millilitre. Use this solution for the preparation of standard stains (*see* **13.3.3**).

13.3 Procedure

13.3.1 *Preparation of the Solution* — Weigh accurately 50 to 100 g of the composite sample (**3.3.2**), transfer to a 500-ml Kjeldahl digestion flask and wet with 25 to 50 ml of concentrated nitric acid. Add 10 to 20 ml of concentrated sulphuric acid and heat cautiously. Add concentrated nitric acid dropwise from time to time from a pipette to speed up the oxidation of the material. Note the total amount of concentrated sulphuric acid and nitric acid added. When the oxidation is complete and the solution is colourless, add 20 ml of water and again boil to fuming. Cool and dilute with water to 50 ml in a graduated flask. Use a suitable aliquot for the estimation of arsenic and reserve the rest for the determination of lead, copper, zinc and tin.

13.3.2 Place an aliquot of the prepared solution (not exceeding 30 ml) estimated to contain 0·01 to 0·03 mg of arsenic in the 60-ml bottle. Add sufficient concentrated hydrochloric acid so as to have a total volume of 5 ml of hydrochloric acid in the aliquot. Cool, if necessary, and add 5 ml of potassium iodide solution and 4 drops of stannous chloride solution. Add 2 to 5 g of granulated zinc, put in the strip of the mercuric bromide paper in the narrow tube and immerse the whole apparatus in a water-bath kept at 20° to 25°C within 2·5 cm of the top of the narrow tube for 1½ hours.

13.3.3 Remove the strip and average the length of the stains on both sides in millimetres. Locate the length of the unknown on a standard graph and read the quantity of arsenic present from the abscissa. The graph is prepared by running known quantities of the standard dilute solution of arsenic (**13.2.9**) by the above method using the length of the stain as ordinate and milligrams of arsenic as abscissa. Carry out a blank determination in case of a positive result.

13.4 Calculation — Calculate the quantity of arsenic present in the material as parts per million.

14. DETERMINATION OF LEAD

14.1 Reagents

14.1.1 *Citric Acid* — solid.

14.1.2 *Ammonium Hydroxide Solution* — sp gr 0·88 or diluted as required.

14.1.3 *Potassium Cyanide Solution* — 10 percent (*w/v*).

14.1.4 *Dithizone (Diphenyl Thiocarbazone) Solution* — 0·1 percent (*w/v*) in chloroform, freshly prepared.

14.1.5 *Dilute Hydrochloric Acid* — approximately 0·1 N.

14.1.6 *Standard Lead Solution* — two reference solutions of lead nitrate are required in this test as given under **14.1.6.1** and **14.1.6.2**.

14.1.6.1 *Standard strong lead solution* — obtained by dissolving 0·160 0 g of lead nitrate [$Pb(NO_3)_2$] in 50 ml of dilute nitric acid and making up the volume in a 100-ml graduated flask.

14.1.6.2 *Standard dilute lead solution* — freshly prepared before the test by diluting one millilitre of the standard strong lead solution to 100 ml with water in a graduated flask.

14.1.7 *Ammonium Acetate* — solid.

14.1.8 *Sodium Sulphide Solution* — 10 percent (*w/v*).

14.2 Procedure

14.2.1 Take a suitable aliquot of the solution (**13.3.1**), add 2 g of citric acid and just neutralize with ammonia. Add one millilitre of the potassium cyanide solution and transfer the whole to a separating funnel. Extract the liquid with the dithizone solution. Carry out 3 extractions, using 10 ml for the first extraction and 5 ml each for the subsequent extractions. If the last extraction gives any indication of a reddish tinge, extract again to ensure complete removal of lead.

14.2.2 Take 10 ml of water in another separating funnel and wash each extract with this water. If suspended matter is present in the chloroform extract, this shall be filtered before passing to the separating funnel containing the 10 ml of wash-water. Transfer the combined chloroform extracts to a separating funnel and extract lead by shaking successively with 50 ml, 20 ml and 10 ml of dilute hydrochloric acid. Combine the acid extracts in a separating funnel, wash once or twice with 10 ml of chloroform and filter through a previously wetted filter paper into a 100-ml graduated flask. Make up the volume of the filtrate to 100 ml with dilute hydrochloric acid and use this as the test solution.

14.2.3 Estimate colorimetrically the lead present by comparison with the standard dilute lead solution containing 0·000 01 g of lead per millilitre (using not more than 10 ml of the standard solution for matching) in the following manner:

> Transfer a suitable volume of the test solution to a Nessler cylinder. Add 2 g of ammonium acetate, followed by ammonia until just alkaline and then one millilitre of potassium cyanide solution. Dilute to 50 ml, add 2 drops of sodium sulphide solution and match the colour against a set of standards prepared in the same way.

14.2.4 A blank determination shall be run under the same conditions, on the same reagents and by the same person but without using the material.

15. DETERMINATION OF COPPER

15.1 Apparatus

15.1.1 *Spectrophotometer*

15.2 Reagents

15.2.1 *Citric Acid* — solid.

15.2.2 *Ammonium Hydroxide Solution* — sp gr 0·90.

15.2.3 *Sodium Diethyldithiocarbamate Solution* — 0·1 percent (*w/v*), aqueous.

15.2.4 *Carbon Tetrachloride* — redistilled.

15.2.5 *Sodium Sulphate* — anhydrous.

15.2.6 *Dilute Nitric Acid* — concentrated nitric acid of sp gr 1·42 diluted with an equal volume of water.

15.2.7 *Standard Copper Solution* — Weigh accurately 0·100 0 g of pure copper turnings, carefully dissolve in the minimum amount of nitric acid, cool and dilute to one litre in a graduated flask. Pipette 10 ml of this solution into a 100-ml graduated flask and dilute to the mark. This solution contains 10 micrograms of copper per millilitre.

15.3 Procedure

15.3.1 Transfer a 10-ml aliquot of the test solution (**13.3.1**) to a separating funnel. Add one gram of nitric acid and dissolve it by shaking. Make the solution alkaline to litmus by adding ammonium hydroxide solution in small quantities. Add 5 ml of the sodium diethyldithiocarbamate solution, shake thoroughly and extract with 5-ml portions of carbon tetrachloride until the final extract is colourless. Dry the combined extracts by shaking thoroughly with anhydrous sodium sulphate. Filter the dry extract and wash the filter paper with carbon tetrachloride. Make up the volume of the filtrate to 25 ml with carbon tetrachloride and measure the absorption at 437 mμm by means of the spectrophotometer. Simultaneously, carry out blank determinations on the water and the reagents.

15.3.2 Prepare a series of standards by treating aliquots of the standard copper solution (**15.2.7**) in the same manner as the test solution. From the absorption of the standard solutions, prepare a standard curve plotting absorption values against concentrations. From the curve, obtain the weight of copper present in the test solution.

16. DETERMINATION OF ZINC

16.1 Apparatus

16.1.1 *Spectrophotometer*

16.2 Reagents

16.2.1 *Methyl Red Indicator Solution* — one percent (*w/v*), aqueous.

16.2.2 *Copper Sulphate Solution* — Dissolve 8 g of copper sulphate ($CuSO_4$, $5H_2O$) in water and dilute to one litre. One millilitre of this solution contains 2 mg of copper.

16.2.3 *Ammonium Hydroxide Solution* — redistilled, sp gr 0·90.

16.2.4 *Concentrated Hydrochloric Acid* — sp gr 1·16.

16.2.5 *Hydrogen Sulphide Gas* — Passed through a wash-bottle containing water.

16.2.6 *Dilute Hydrochloric Acid* — containing 5 percent by weight of concentrated hydrochloric acid.

16.2.7 *Bromine Water* — saturated.

16.2.8 *Phenol Red Indicator Solution* — prepared by dissolving 0·1 g of phenol red in 100 ml of rectified spirit (*see* 323-1959*).

16.2.9 *Hydrochloric Acid* (1 : 1) — Dilute concentrated hydrochloric acid with an equal volume of water.

16.2.10 *Ammonium Citrate Solution* — Dissolve 225 g of ammonium citrate in water, make alkaline to phenol red with ammonium hydroxide and add 75 ml in excess. Dilute to 2 litres. Extract this solution immediately before use as follows:

> Add to the solution a slight excess of dithizone solution and extract with carbon tetrachloride until the solvent layer is clear bright-green. Remove the excess of dithizone by repeated extraction with chloroform and finally extract once more with carbon tetrachloride. (It is essential that excess dithizone be entirely removed, otherwise zinc will be lost during the elimination of cobalt and nickel.)

16.2.11 *Dimethylglyoxime Solution* — Dissolve 2 g of dimethylglyoxime in 10 ml of ammonium hydroxide solution and 200 to 300 ml of water, filter and dilute to one litre.

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*Specification for rectified spirit (*revised*).

16.2.12 *α-Nitroso β-Naphthol Solution* — Dissolve 0·25 g of α-nitroso β-naphthol in chloroform and dilute to 500 ml with chloroform.

16.2.13 *Chloroform* — redistilled.

16.2.14 *Dithizone (Diphenylthiocarbazone) Solution* — Dissolve 0·05 g of dithizone in 2 ml of ammonium hydroxide solution and 100 ml of water and extract repeatedly with carbon tetrachloride until the solvent layer is clear bright-green in colour. Discard the solvent layer and filter the aqueous portion through a washed ashless filter paper. (This solution is best prepared as needed since it is only moderately stable even when kept in the dark and under refrigeration.)

16.2.15 *Carbon Tetrachloride* — redistilled.

16.2.16 *Hydrochloric Acid* — approximately 0·04 N.

16.2.17 *Stock Solution of Zinc* — Dissolve exactly 0·500 g of pure granulated zinc in slight excess of dilute hydrochloric acid and dilute to one litre in a graduated flask.

16.2.18 *Standard Solution of Zinc* — At the time of the experiment, dilute 10 ml of the stock solution of zinc (**16.2.17**) to 1 000 ml with hydrochloric acid (0·04 N). This solution contains 5 micrograms of zinc per millilitre.

16.3 Procedure

16.3.1 *Separation of Sulphide Group* — Take a suitable aliquot of the test solution (**13.3.1**) to contain 25 to 100 micrograms of zinc, add 2 drops of methyl red indicator solution, one millilitre of copper sulphate solution and neutralize with ammonium hydroxide solution. Add sufficient quantity of concentrated hydrochloric acid to make the solution about 0·15 N with respect to this acid (about 0·75 ml excess in 50 ml of solution is satisfactory). The *p*H of the solution at this point should be 1·9 to 2·1 when measured with a glass electrode. Pass a stream of hydrogen sulphide gas into the solution until precipitation is complete. Filter the contents of the flask through a fine textured filter paper (Whatman No. 42 or equivalent) that has been previously fitted into the funnel and washed first with dilute hydrochloric acid and then with water. Collect the filtrate in a beaker and wash the flask and the filter with 3 to 4 small portions of water. Boil the filtrate gently until the odour of hydrogen sulphide is no longer detected; then add 5 ml of bromine water and continue boiling until bromine has been expelled. Allow the solution to cool, neutralize with ammonium hydroxide solution using phenol red as indicator and then make it slightly acidic with hydrochloric acid (1 : 1) by adding an excess of 0·2 ml. Dilute the resultant solution in a graduated flask to contain 0·2 ml to 1·0 microgram of zinc per millilitre.

16.3.2 *Elimination of Nickel and Cobalt* — Transfer a 20 ml aliquot of the solution (obtained under **16.3.1**) to a separating funnel. Add to it 5 ml of ammonium citrate solution, 2 ml of dimethylglyoxime solution and 10 ml of α-nitroso β-naphthol solution and shake the contents of the funnel for two minutes. Discard the lower layer and extract the aqueous layer with 10 ml of chloroform to remove residual α-nitroso β-naphthol. Discard the chloroform layer.

16.3.3 *Isolation and Estimation of Zinc*

16.3.3.1 To the aqueous layer obtained after eliminating nickel and cobalt (**16.3.2**) which at this point has a *p*H of 8·0 to 8·2, add 2·0 ml of the dithizone solution and 10 ml of carbon tetrachloride, and shake for two minutes. Allow the layers to separate and remove the aqueous layer as completely as possible, withdrawing it by means of a pipette attached to the vacuum line. Wash down the sides of the separating funnel with about 25 ml of water and draw off the aqueous layer without shaking. Add 25 ml of hydrochloric acid (0·04 N) to the carbon tetrachloride layer in the separating funnel and shake for one minute to transfer the zinc to the acid-aqueous layer. Drain off and discard the carbon tetrachloride layer taking care to dislodge and remove the drop that usually floats on the surface. To the acid aqueous layer, add 5·0 ml of ammonium citrate solution and 10·0 ml of carbon tetrachloride (*p*H of the solution at this point is 8·8 to 9·0). Add the requisite volume of the dithizone solution calculated as follows:

> Pipette 4·0 ml of the standard solution of zinc into a separating funnel, add to it 21 ml of hydrochloric acid (0·04 N) from a burette, 5 ml of ammonium citrate solution, 10·0 ml of carbon tetrachloride and then add the dithizone solution in 0·1 ml increments, shaking briefly after each addition until a faint yellow colour in the aqueous layer indicates a bare excess of the reagent. Note the total volume of the dithizone solution added. Multiply this volume by 1·5.

After the addition of dithizone solution, shake the separating funnel for two minutes. Pipette 5·0 ml of the carbon tetrachloride layer and transfer it to the spectrophotometer cell. Dilute the solution with 10·0 ml of carbon tetrachloride, mix and determine its transmission at 540 mμm.

NOTE — Dilution may be made in a clean dry test-tube if the design of the cell does not permit direct mixing.

16.3.3.2 Pipette into a series of separating funnels 0, 1, 2, 3 and 4 ml of the standard solution of zinc and add the necessary volume of hydrochloric acid (0·04 N) to make 25 ml. Add into each separator 5·0 ml of the ammonium citrate solution and the calculated volume of the dithizone solution. Shake the separating funnel, pipette out 5·0 ml of the carbon tetrachloride layer and transfer it to the spectrophotometer cell. Dilute the solution with 10·0 ml of carbon tetrachloride, mix and determine its transmission at 540 mμm. Proceed in the same manner with the solutions contained in other separating funnels.

16.3.3.3 Plot the transmittance for each of the series of separating funnels on logarithmic scale against concentration of zinc in micrograms present in 25 ml of the diluted standard zinc solution in the particular separating funnel and draw a smooth curve through the points. (Intercept of this curve may vary from day to day depending on the actual concentration of dithizone used in the final extraction, but the slope of the curve should remain essentially the same.) From this curve, obtain the weight of zinc in micrograms.

17. DETERMINATION OF TIN

17.1 Reagents

17.1.1 *Ammonium Hydroxide Solution* — sp gr 0·90.

17.1.2 *Concentrated Hydrochloric Acid* — sp gr 1·16.

17.1.3 *Dilute Sulphuric Acid* — 1 : 3 by volume.

17.1.4 *Hydrogen Sulphide Gas* — passed through a wash-bottle containing water.

17.1.5 *Wash Solution* — Mix 100 ml of saturated ammonium acetate solution with 50 ml of glacial acetic acid and 850 ml of water.

17.1.6 *Ammonium Polysulphide Solution* — Pass hydrogen sulphide gas through 200 ml of ammonium hydroxide solution contained in a bottle immersed in ice-cold water until no more gas is absorbed, add 200 ml of the ammonium hydroxide solution and dilute with water to make 1 000 ml. Add 25 g of flowers of sulphur to this solution and keep for several hours to digest the sulphur and then filter. Use the filtrate.

17.1.7 *Dilute Acetic Acid* — Dilute one volume of glacial acetic acid with 9 volumes of water.

17.2 Procedure

17.2.1 Take a suitable aliquot of the test solution (**13.3.1**), dilute to 400 ml, cool and add ammonium hydroxide solution until the contents are alkaline. Add 20 ml of either concentrated hydrochloric acid or dilute sulphuric acid. Cover the beaker with a watch-glass. Heat the solution to about 95°C and pass a slow stream of hydrogen sulphide gas through it for one hour. Digest the mixture at 95°C for one hour and allow it to stand for another 30 minutes. Filter the contents of the beaker through a quantitative filter paper and wash the residue of stannous sulphide on the filter, alternatively with three portions each of wash solution and hot water. Transfer the residue along with the filter paper to a 50-ml beaker, add 10 to 20 ml of the ammonium polysulphide solution, heat to boiling and filter by decantation. Add 10 ml of the ammonium polysulphide solution, boil and filter again. Repeat this once more and finally wash the filter paper with hot water. Acidify the combined filtrate and washings with dilute acetic acid, gently boil for one hour and allow to stand overnight. Filter the resulting mixture through a double 11-cm ashless filter paper. Wash the filter paper alternately with two portions each of the wash solution and hot water. Transfer the residue along with the filter paper to a tared porcelain crucible and dry it thoroughly in an air-oven. Carefully ignite the filter paper, using a Bunsen flame and incinerate the contents to convert the sulphide to oxide. Partly cover the crucible and heat strongly over a Bunsen or Meker burner. (The sulphide shall be gently roasted to the oxide which may then be heated to a high temperature without loss by volatilization.)

17.2.2 Cool the crucible in a desiccator and weigh as stannous oxide (SnO_2). Repeat the process of heating, cooling and weighing till the difference between two successive weighings is less than one milligram. Note the lowest weight. Obtain the weight of metallic tin from the weight of stannous oxide by using the factor 0·787 7.

18. TEST FOR MICROBIOLOGICAL REQUIREMENTS

18.1 General — The test consists of the incubation of the cans at 37°C and 55°C for not less than 7 days and their examination at the end of this period for evidence of microbiological activity. To satisfy the requirements of this test, the cans shall not show evidence of microbiological activity of any type. The incubated cans may have undergone microbiological spoilage if any can:

a) shows a positive pressure;

b) bulges when being incubated and remains bulged after being cooled, or remains bulged after incubation; and

c) leaks.

18.1.1 Whether the cans show a positive pressure or not, the evidence of bacterial proliferation shall be judged by:

a) a significant change in *p*H value,

b) disintegration or digestion of the fruit or vegetable as compared with the unincubated samples,

c) microscopic examination of direct smears, and

d) culture methods for testing bacterial multiplication and presence of pathogenic bacteria.

18.2 Apparatus

18.2.1 *Glassware* — All the glass apparatus used in the microbiological examination shall be sterile. Sterilization shall be performed for at least two hours at 170°C in dry heat.

18.2.2 *Accessories* — Can-opener of the puncturing type, cork borers, sampling tubes, and cotton wool — all sterile.

18.3 Reagents

18.3.1 *Petroleum Ether*

18.3.2 *Mercuric Chloride Solution* — 1·0 percent (*w*/*v*).

18.3.3 *Carbolic Acid Solution* — 1·5 percent (*w*/*v*).

18.3.4 *Ethyl Alcohol Solutions* — 97 percent, 95 percent and 98 percent (*w*/*v*).

18.3.5 *Chloroform*

18.3.6 *Formaldehyde* — 40 percent (*w*/*v*).

18.3.7 *Sterile Normal Saline or Ringers Solution*

18.3.7.1 *Normal saline* — 0·9 percent (*w*/*v*) solution of sodium chloride (analytical reagent).

18.3.7.2 *Ringers solution* — It has the following composition:

Sodium chloride	0·2 g
Potassium chloride	0·42 g
Calcium chloride	0·48 g
Sodium carbonate	0·2 g
Distilled water	1 000 ml (glass distilled)

18.3.7.3 Ringers solution is diluted to quarter strength when actually in use. The sterilization of the normal saline and the Ringers solution shall be performed at 7 kg pressure at 121°C for 20 minutes in an autoclave.

18.3.8 *Sterile Vaseline* — Alternatively, sterile liquid paraffin, liquid agar or paraffin wax may be used.

18.4 Media

18.4.1 *Dextrose-Tryptone-Broth* — Mix 10 g of tryptone, 5 g of dextrose, 0·04 g of lexomo-cresol-purple and one litre of water. Steam the mixture until dissolved. Adjust the *p*H to 6·8 to 7·0. Filter and tube in 10-ml amounts and autoclave at 121°C for 30 minutes. This media is used for detecting the growth of thermophilic anærobes not producing hydrogen sulphide, putrefactive anærobes and other mesophilic anærobes.

18.4.2 *Liver Broth* — Boil 500 g of well-minced degutted beef liver in 1 000 ml of distilled water for one hour. Adjust the *p*H of the mixture to 7·0 and boil further for 10 minutes. Cool and separate all fat by straining through several layers of muslin and make up the volume to one litre with water. Add 10 g of peptone, one gram of dipotassium hydrogen phosphate (K_2HPO_4) and again adjust the *p*H to 7·0, tube in 10-ml quantities, add to each tube 2 g of liver particles and autoclave at 121°C for 30 minutes. Before use, boil the medium for 10 to 15 minutes to remove dissolved air. Cool the tube before inoculation. The medium may further be improved by sealing with paraffin wax or liquid paraffin.

18.4.3 *Cooked Meat Medium* — Mince 500 g of fresh bullock's heart, place in 500 ml of boiling distilled water to which has been added 1·5 ml of 1N sodium hydroxide solution and simmer for twenty minutes, by the end of which the neutralization of lactic acid will be ensured. Drain off the liquid through a muslin filter and, while still hot, press the minced meat in a cloth and dry partially by spreading it on a cloth or filter paper. In this condition it may be introduced into bottles without boiling them. Place about 2·5 g of the minced meat in each bottle and cover with 10 ml of peptone infusion broth made as follows:

> Add peptone 0·5 percent and sodium chloride 0·25 percent to the liquid filtered from the meat. Steam for 20 minutes, add 1·0 ml of concentrated hydrochloric acid and filter. Bring the reaction of the filtrate to *p*H 7·7 to 7·8. Autoclave at 120°C for twenty minutes. After sterilization, the *p*H of the broth over the meat is 7·4 to 7·5. Introduce the inoculum towards the bottom of the tube in contact with the meat. Cooked meat medium is used for detecting the presence of spoilage bacteria in acid foods like tomato products.

18.4.4 *Nutrient-Agar* — prepared from the following ingredients:

Polypeptone (peptone)	5 g
Beef extract	3 g
Agar	15 g
Water	1 000 ml
*p*H	6·2 to 7·0

Sterilize the media at 121°C for 15 minutes. Nutrient-agar is used for the isolation of organisms.

18.4.5 *Potato-Dextrose-Agar* — prepared from the following ingredients:

Infusion from 200 g potato	1 000 ml
Glucose	20 g
Agar	15 g

Sterilize the media at 121°C for 15 minutes. Adjust the *p*H to 3·5 ± 0·1 at the time of pouring into plates. Potato-dextrose-agar is used for the growth of yeasts and moulds, and their counts.

18.4.6 *Malt-Extract-Agar* — prepared from the following ingredients:

Malt extract	45 g
Agar	15 g
Water	1 000 ml

Sterilize the media at 121°C for 15 minutes. Adjust the *p*H to 3·5 ± 0·1 at the time of pouring into plates. This media is used for the growth of yeasts and moulds.

18.5 Procedure

18.5.1 *Preparation of the Can*

18.5.1.1 Note down all the identification marks, brand marks, batch number, date and other information available on the label or embossed on the tin. Remove the label and record all physical defects, such as rust, pin-holing, dents, signs or defective seaming of end-plates and of defective side seams. Note other points, if any, for examination after the can is opened.

18.5.1.2 Scrub the can with soap and water. If necessary, apply petroleum ether and clean. After washing with soap, immerse for at least one hour in a tall container filled with mercuric chloride solution or carbolic acid solution.

18.5.1.3 After removing from the antiseptic bath, clean the exterior of the can with sterile cotton wool. When dry, scrub well with sterile cotton wool dipped in ethyl alcohol (97 percent). Cover the top of the can with sterile petri dish.

18.5.2 *Preparation of the Sample for Bacteriological Tests* — Puncture the can and enlarge the puncture under aseptic conditions with a thoroughly sterilized can-opener to give an opening of 2·5 cm diameter. If a cutting type of instrument is used, cut a triangle with a base of 2·5 cm and sides 5 cm long. Cover the enlarged opening at once with sterile petri dish.

18.5.3 *Removal of the Inoculum* (*Sample*)

18.5.3.1 *Removal of liquid inoculum* — The inoculum is preferably removed by means of a sterile pipette graduated in millilitres. It is essential that the rear end of the pipette be plugged for a length not less than 1·5 cm with sterile cotton wool. Deliver 2 ml of the inoculum to each of the broth tubes and one millilitre to each of the melted agar tubes. Mix well to ensure uniform distribution and pour into the petri dishes swirling so as to obtain uniform spread. Alternatively, pour 2 ml of the inoculum into the petri dish and then add the melted agar medium and mix by swirling.

18.5.3.2 *Removal of solid inoculum* — For removing the samples of solid material, use sterile cork borers. Remove cores of the pack from the centre and side regions of the can. Remove about 15 g of the sample and transfer it to a sterile flask containing 50 ml of sterile water or sterile normal saline or sterile Ringers solution. The flask should contain at least a dozen sterile glass beads to facilitate mixing. Mix by swirling for at least 5 minutes. Take out 2-ml portions of the inoculum and add them to both the broths and the agars.

18.5.4 *Preparation of the Anærobic Culture* — The dextrose-tryptone-broth and the liver-broth media are rendered anærobic by sealing the access to air by placing a layer of sterile vaseline, liquid paraffin, liquid agar or paraffin wax on the top of the medium. This layer should be not less than 1·5 cm in thickness. Just before using the culture tubes, exhaust the media by packing the tubes for at least 10 minutes in a water-bath and maintain the water-bath at 70°C. Cool the media to 37°C and inoculate the medium below the paraffin agar layer with as little disturbance as possible and cool the medium after inoculation in a cold water-bath so that the top seal is set.

18.5.5 *Incubation*

18.5.5.1 Incubate the inoculated tubes and petri dishes as follows:

a) *Dextrose-tryptone-broth* — two tubes each, ærobically and anærobically, at 37°C for 3 to 5 days and similarly another set at 55°C for 3 to 5 days.

b) *Liver broth* — two tubes anærobically at 37°C and two tubes at 55°C for 5 days.

c) *Malt-extract-broth* — two tubes ærobically, one at 25°C and the other at 37°C for 5 days.

d) *Cooked meat medium* — one tube anærobically at 37°C for 5 days.

e) *Potato-dextrose-agar or malt-extract-agar* — two dishes ærobically, one at 25°C and the other at 37°C for 5 days.

18.5.5.2 In the case of a blown can, run a separate series of culture tubes parallel to the tubes from the sound can. The incubation period is the same as given in **18.5.5.1**.

18.5.6 *Examination of Culture After Incubation*

18.5.6.1 In case there is growth in the nutrient-broth tubes, streak them on agar plates and incubate. Examine the contents of each culture tube for the organisms of the following types:

a) Spoilage types,

b) Potential spoilage types, and

c) Pathogenic types.

18.5.6.2 In case there is a growth of cocci, establish their nature by streaking them on blood agar and study ability for hæmolysis and nature by (a) coagulase tests, and (b) by microscopic examination.

18.5.7 *Examination of the Cans After the Removal of the Inoculum from the Cans* — Carry out the following tests:

a) Prepare a direct smear of the contents. Stain by Gram's method or methyl violet and examine the preparations microscopically;

b) Determine the *p*H value;

c) Examine contents for deterioration, discolouration, disintegration and other signs of spoilage;

d) The tinplate should be examined for nature of etching, staining or corrosion disturbance of lacquer and rust; and

e) The seams should be tested for leaks and soundness of fabrication.

18.5.7.1 Correlate the above findings with the incidence and nature of microflora and conclude about the cause of the spoilage, if any.

APPENDIX A

(*Clause* 9.3.2.1)

DEGREES BRIX, SPECIFIC GRAVITY AND DEGREES BAUME OF SUGAR SOLUTIONS

Degrees Brix or Percent by Weight of Sucrose	Specific Gravity at 20°/20°C	Specific Gravity at 20°/4°C	Degrees Baume (Modulus 145)
0·0	1·000 00	0·998 234	0·00
0·2	1·000 78	0·999 010	0·11
0·4	1·001 55	0·999 786	0·22
0·6	1·002 33	1·000 563	0·34
0·8	1·003 11	1·001 342	0·45
1·0	1·003 89	1·002 120	0·56
1·2	1·004 67	1·002 897	0·67
1·4	1·005 45	1·003 675	0·79
1·6	1·006 23	1·004 453	0·90
1·8	1·007 01	1·005 234	1·01
2·0	1·007 79	1·006 015	1·12
2·2	1·008 58	1·006 796	1·23
2·4	1·009 36	1·007 580	1·34
2·6	1·010 15	1·008 363	1·46
2·8	1·010 93	1·009 148	1·57
3·0	1·011 72	1·009 934	1·68
3·2	1·012 51	1·010 721	1·79
3·4	1·013 30	1·011 510	1·90
3·6	1·014 09	1·012 298	2·02
3·8	1·014 88	1·013 089	2·13

(*Continued*)

DEGREES BRIX OR PERCENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20°/20°C	SPECIFIC GRAVITY AT 20°/4°C	DEGREES BAUMÉ (MODULUS 145)
4·0	1·015 67	1·013 881	2·24
4·2	1·016 47	1·014 673	2·35
4·4	1·017 26	1·015 467	2·46
4·6	1·018 06	1·016 261	2·57
4·8	1·018 86	1·017 058	2·68
5·0	1·019 65	1·017 854	2·79
5·2	1·020 45	1·018 652	2·91
5·4	1·021 25	1·019 451	3·02
5·6	1·022 06	1·020 251	3·13
5·8	1·022 86	1·021 053	3·24
6·0	1·023 66	1·021 855	3·35
6·2	1·024 47	1·022 659	3·46
6·4	1·025 27	1·023 463	3·57
6·6	1·026 08	1·024 270	3·69
6·8	1·026 89	1·025 077	3·80
7·0	1·027 70	1·025 885	3·91
7·2	1·028 51	1·026 694	4·02
7·4	1·029 32	1·027 504	4·13
7·6	1·030 13	1·028 316	4·24
7·8	1·030 95	1·029 128	4·35
8·0	1·031 76	1·029 942	4·46
8·2	1·032 58	1·030 757	4·58
8·4	1·033 40	1·031 573	4·69
8·6	1·034 22	1·032 391	4·80
8·8	1·035 04	1·033 209	4·91
9·0	1·035 86	1·034 029	5·02
9·2	1·036 68	1·034 850	5·13
9·4	1·037 50	1·035 671	5·24
9·6	1·038 33	1·036 494	5·35
9·8	1·039 15	1·037 318	5·46
10·0	1·039 98	1·038 143	5·57
10·2	1·040 81	1·038 970	5·68
10·4	1·041 64	1·039 797	5·80
10·6	1·042 47	1·040 626	5·91
10·8	1·043 30	1·041 456	6·02
11·0	1·044 13	1·042 288	6·13
11·2	1·044 97	1·043 121	6·24
11·4	1·045 80	1·043 954	6·35
11·6	1·046 64	1·044 788	6·46
11·8	1·047 47	1·045 625	6·57
12·0	1·048 31	1·046 462	6·68
12·2	1·049 15	1·047 300	6·79
12·4	1·049 99	1·048 140	6·90
12·6	1·050 84	1·048 980	7·02
12·8	1·051 68	1·049 322	7·13

(*Continued*)

Degrees Brix or Percent by Weight of Sucrose	Specific Gravity at 20°/20°C	Specific Gravity at 20°/4°C	Degrees Baume (Modulus 145)
13·0	1·052 52	1·050 665	7·24
13·2	1·053 37	1·051 510	7·35
13·4	1·054 22	1·052 356	7·46
13·6	1·055 06	1·053 202	7·57
13·8	1·055 91	1·054 050	7·68
14·0	1·056 77	1·054 900	7·79
14·2	1·057 62	1·055 751	7·90
14·4	1·058 47	1·056 602	8·01
14·6	1·059 33	1·057 455	8·12
14·8	1·060 18	1·058 310	8·23
15·0	1·061 04	1·059 165	8·34
15·2	1·061 90	1·060 022	8·45
15·4	1·062 76	1·060 880	8·56
15·6	1·063 62	1·061 738	8·67
15·8	1·064 48	1·062 598	8·78
16·0	1·065 34	1·063 460	8·89
16·2	1·066 21	1·064 324	9·00
16·4	1·067 07	1·065 188	9·11
16·6	1·067 94	1·066 054	9·22
16·8	1·068 81	1·066 921	9·33
17·0	1·069 68	1·067 789	9·45
17·2	1·070 55	1·068 658	9·56
17·4	1·071 42	1·069 529	9·67
17·6	1·072 29	1·070 400	9·78
17·8	1·073 17	1·071 273	9·89
18·0	1·074 04	1·072 147	10·00
18·2	1·074 92	1·073 023	10·11
18·4	1·075 80	1·073 900	10·22
18·6	1·076 68	1·074 777	10·33
18·8	1·077 56	1·075 657	10·44
19·0	1·078 44	1·076 537	10·55
19·2	1·079 32	1·077 419	10·66
19·4	1·080 21	1·078 302	10·77
19·6	1·081 10	1·079 187	10·88
19·8	1·081 98	1·080 072	10·99
20·0	1·082 87	1·080 959	11·10
20·2	1·083 76	1·081 848	11·21
20·4	1·084 65	1·082 737	11·32
20·6	1·085 54	1·083 628	11·43
20·8	1·086 44	1·084 520	11·54
21·0	1·087 33	1·085 414	11·65
21·2	1·088 23	1·086 309	11·76
21·4	1·089 13	1·087 205	11·87
21·6	1·090 03	1·088 101	11·98
21·8	1·090 93	1·089 000	12·09

(*Continued*)

DEGREES BRIX OR PERCENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20°/20 C	SPECIFIC GRAVITY AT 20°/4 C	DEGREES BAUME (MODULUS 145)
22·0	1·091 83	1·089 900	12·20
22·2	1·092 73	1·090 802	12·31
22·4	1·093 64	1·091 704	12·42
22·6	1·094 54	1·092 607	12·52
22·8	1·095 45	1·093 513	12·63
23·0	1·096 36	1·094 420	12·74
23·2	1·097 27	1·095 328	12·85
23·4	1·098 18	1·096 236	12·96
23·6	1·099 09	1·097 147	13·07
23·8	1·100 00	1·098 058	13·18
24·0	1.100 92	1·098 971	13·29
24·2	1·101 83	1·099 886	13·40
24·4	1·102 75	1·100 802	13·51
24·6	1·103 67	1·101 718	13·62
24·8	1·104 59	1·102 637	13·73
25·0	1·105 51	1·103 557	13·84
25·2	1·106 43	1·104 478	13·95
25·4	1·107 36	1·105 400	14·06
25·6	1·108 28	1·106 324	14·17
25·8	1·109 21	1·107 248	14·28
26·0	1·110 14	1·108 175	14·39
26·2	1·111 06	1·109 103	14·49
26·4	1·112 00	1·110 033	14·60
26·6	1·112 93	1·110 963	14·71
26·8	1·113 86	1·111 895	14·82
27·0	1·114 80	1·112 828	14·93
27·2	1·115 73	1·113 763	15·04
27·4	1·116 67	1·114 697	15·15
27·6	1·117 61	1·115 635	15·26
27·8	1·118 55	1·116 572	15·37
28·0	1·119 49	1·117 512	15·48
28·2	1·120 43	1·118 453	15·59
28·4	1·121 38	1·119 395	15·69
28·6	1·122 32	1·120 339	15·80
28·8	1·123 27	1·121 284	15·91
29·0	1·124 22	1·122 231	16·02
29·2	1·125 17	1·123 179	16·13
29·4	1·126 12	1·124 128	16·24
29·6	1·127 07	1·125 079	16·35
29·8	1·128 02	1·126 030	16·46
30·0	1·128 98	1·126 984	16·57
30·2	1·129 93	1·127 939	16·67
30·4	1·130 89	1·128 896	16·78
30·6	1·131 85	1·129 853	16·89
30·8	1·132 81	1·130 812	17·00

(*Continued*)

DEGREES BRIX OR PERCENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20°/20°C	SPECIFIC GRAVITY AT 20°/4°C	DEGREES BAUME (MODULUS 145)
31·0	1·133 78	1·131 773	17·11
31·2	1·134 74	1·132 735	17·22
31·4	1·135 70	1·133 698	17·33
31·6	1·136 67	1·134 663	17·43
31·8	1·137 64	1·135 628	17·54
32·0	1·138 61	1·136 596	17·65
32·2	1·139 58	1·137 565	17·76
32·4	1·140 55	1·138 534	17·87
32·6	1·141 52	1·139 506	17·96
32·8	1·142 50	1·140 479	18·08
33·0	1·143 47	1·141 453	18·19
33·2	1·144 45	1·142 429	18·30
33·4	1·145 43	1·143 405	18·41
33·6	1·146 41	1·144 384	18·52
33·8	1·147 39	1·145 363	18·63
34·0	1·148 37	1·146 345	18·73
34·2	1·149 36	1·147 328	18·84
34·4	1·150 34	1·148 313	18·95
34·6	1·151 33	1·149 298	19·06
34·8	1·152 32	1·150 286	19·17
35·0	1·153 31	1·151 275	19·28
35·2	1·154 30	1·152 265	19·38
35·4	1·155 30	1·153 256	19·49
35·6	1·156 29	1·154 249	19·60
35·8	1·157 29	1·155 242	19·71
36·0	1·158 28	1·156 238	19·81
36·2	1·159 28	1·157 235	19·92
36·4	1·160 28	1·158 233	20·03
36·6	1·161 28	1·159 233	20·14
36·8	1·162 28	1·160 233	20·25
37·0	1·163 29	1·161 236	20·35
37·2	1·164 30	1·162 240	20·46
37·4	1·165 30	1·163 245	20·57
37·6	1·166 31	1·164 252	20·68
37·8	1·167 32	1·165 259	20·78
38·0	1·168 33	1·166 269	20·89
38·2	1·169 34	1·167 281	21·00
38·4	1·170 36	1·168 293	21·11
38·6	1·171 38	1·169 307	21·21
38·8	1·172 39	1·170 322	21·32
39·0	1·173 41	1·171 340	21·43
39·2	1·174 43	1·172 359	21·54
39·4	1·175 45	1·173 379	21·64
39·6	1·176 48	1·174 400	21·75
39·8	1·177 50	1·175 423	21·86

(*Continued*)

DEGREES BRIX OR PERCENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20°/20°C	SPECIFIC GRAVITY AT 20°/4°C	DEGREES BAUME (MODULUS 145)
40·0	1·178 53	1·176 447	21·97
40·2	1·179 56	1·177 473	22·07
40·4	1·180 58	1·178 501	22·18
40·6	1·181 62	1·179 527	22·29
40·8	1·182 65	1·180 560	22·39
41·0	1·183 68	1·181 592	22·50
41·2	1·184 72	1·182 625	22·61
41·4	1·185 75	1·183 660	22·72
41·6	1·186 79	1·184 696	22·82
41·8	1·187 83	1·185 734	22·93
42·0	1·188 87	1·186 773	23·04
42·2	1·189 92	1·187 814	23·14
42·4	1·190 96	1·188 856	23·25
42·6	1·192 01	1·189 901	23·36
42·8	1·193 05	1·190 946	23·46
43·0	1·194 10	1·191 993	23·57
43·2	1·195 15	1·193 041	23·68
43·4	1·196 20	1·194 090	23·78
43·6	1·197 26	1·195 141	23·89
43·8	1·198 31	1·196 193	24·00
44·0	1·199 36	1·197 247	24·10
44·2	1·200 42	1·198 303	24·21
44·4	1·201 48	1·199 360	24·32
44·6	1·202 54	1·200 420	24·42
44·8	1·203 60	1·201 480	24·53
45·0	1·204 67	1·202 540	24·63
45·2	1·205 73	1·203 603	24·74
45·4	1·206 80	1·204 668	24·85
45·6	1·207 87	1·205 733	24·95
45·8	1·208 94	1·206 801	25·06
46·0	1·210 01	1·207 870	25·17
46·2	1·211 08	1·208 940	25·27
46·4	1·212 15	1·210 013	25·38
46·6	1·213 23	1·211 086	25·48
46·8	1·214 31	1·212 162	25·59
47·0	1·215 38	1·213 238	25·70
47·2	1·216 46	1·214 317	25·80
47·4	1·217 55	1·215 395	25·91
47·6	1·218 63	1·216 476	26·01
47·8	1·219 71	1·217 559	26·12
48·0	1·220 80	1·218 643	26·23
48·2	1·221 89	1·219 729	26·33
48·4	1·222 98	1·220 815	26·44
48·6	1·224 06	1·221 904	26·54
48·8	1·225 16	1·222 995	26·65

(Continued)

Degrees Brix or Percent by Weight of Sucrose	Specific Gravity at 20°/20°C	Specific Gravity at 20°/4°C	Degrees Baume (Modulus 145)
49·0	1·226 25	1·224 086	26·75
49·2	1·227 35	1·225 180	26·86
49·4	1·228 44	1·226 274	26·96
49·6	1·229 54	1·227 371	27·07
49·8	1·230 64	1·228 469	27·18
50·0	1·231 74	1·229 567	27·28
50·2	1·232 84	1·230 668	27·39
50·4	1·233 95	1·231 770	27·49
50·6	1·235 06	1·232 874	27·60
50·8	1·236 16	1·233 979	27·70

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